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Let's talk about stress, baby! Infant-feeding practices and stress in the ancient Atacama Desert, northern Chile.

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ABSTRACT

Aims and objectives: The transition to an agricultural economy is often presumed to involve an increase in female fertility related to changes in weaning practice. In particular, the availability of staple crops as complementary foods is hypothesized to allow earlier weaning in agricultural populations. In this study our primary aim is to explore whether this model fits the agricultural transition in the Atacama Desert using incremental isotopic analysis. A secondary aim of this study is to identify isotopic patterns relating to weaning, and assess how these may be differentiated from those relating to early life stress.

Materials and Methods: We use incremental isotopic analysis of dentine to examine changes in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from infancy and childhood in sites of the Arica region ($n = 30$). We compare individuals from pre-agricultural and agricultural phases to establish isotopic patterns and relate these patterns to maternal diet, weaning trajectory and physiological stress.

Results: We find that there is no evidence for systematic temporal or geographic variation in incremental isotopic results. Instead, results from all time periods are highly variable, with weaning completed between 1.5 and 3.5 years. Characteristics of the incremental profiles indicate that both *in utero* and postnatal stress were a common part of the infant experience in the Atacama.

Discussion: In the Atacama Desert it appears that the arrival of agricultural crops did not result in uniform shifts in weaning behavior. Instead, infant and child diet seems to have been dictated by the broad-spectrum diets of the mothers, perhaps as a way of mitigating the stresses of the harsh desert environment.

The study of weaning behavior in archaeological contexts is becoming increasingly commonplace. This is due in part to growing acknowledgement that this life-history process has significant sociobiological implications. Changes to weaning behavior may have links to a variety of factors of interest to the bioarchaeologist, including female fertility (Bentley, Paine, & Boldsen, 2001; Jay, 2009), the presence of agricultural resources which may be used as complementary foods (Buikstra, Konigsberg, & Bullington, 1986), infant and maternal health (Lawrence & Lawrence, 2010; Lewis, 2007; Stuart-Macadam, 1995) and changes to cultural norms and taboos surrounding infant-feeding (Sellen & Smay, 2001; Stuart-Macadam, 1995). The weaning process is also intimately associated with physiological stress, a factor which can have long-lasting implications for population health and potential for growth (Bentley et al., 2001; Bocquet-Appel, 2011; McDade & Worthman, 1998). A suckling infant obtains passive immunity to a number of pathogens through the transfer of antibodies, elements of the intestinal biome, mucosal immunity, nucleotides, probiotics, prebiotics, oligosaccharides and glycans – all of which are important components of the developing immune system (Lawrence & Lawrence, 2010). Exclusively breastfed infants are, therefore, less susceptible to infectious disease (Ladomenou, Moschandreas, Kafatos, Tselentis, & Galanakis, 2010). Beyond the age of around 6 months, however, breastmilk cannot fully meet the energetic and nutritional requirements of the infant, and complementary foods must be introduced to sustain growth (Dewey, 2013; McDade & Worthman, 1998). This introduction of complementary foods has the potential to introduce physiological stress through exposure to pathogens at a point in time when the infant's own immunity is only just developing (Goodman & Armelagos, 1989).

This interaction between infant-feeding and stress means that weaning is much more complex than the discrete life event it is often described as in the archaeological literature. Weaning is a process, beginning when the mother starts to supplement breastmilk with complementary foods and ending when the child stops breastfeeding. Although the timing of weaning events as part of this process may be linked to cultural expectations (Bledsoe & Hill, 1998; Leeming, Williamson, Lyttle, & Johnson, 2013), there are many other factors which feed into decision-making. These include female workload (Nerlove, 1974; Sellen & Smay, 2001), nutritional status, infant or maternal health (Simondon, Costes, Delaunay, Diallo, & Simondon, 2001), and female choice in deliberately allowing or avoiding a further pregnancy (Jelliffe & Jelliffe, 1978; Kennedy, Rivera, & McNeilly, 1989). The contribution of these factors means that weaning is a deeply individual process (Kendall, 2016). In addition, the process of weaning may not be entirely linear - a mother may return to exclusively breastfeeding if, for example, the health of her infant is perceived to be suffering because of the weaning process (Simondon et al., 2001). Similarly, breastfeeding may cease suddenly due to maternal stress caused by inadequate nutrition or disease (Dewey, 2001). The complexity of the weaning process means that it is difficult for bioarchaeologists to tease apart the factors that contributed to a past population's decisions about when and how to wean. It is even more difficult to interpret what changes in weaning behavior may mean for population dynamics and female fertility on a broader-scale.

In recent years, the development of incremental isotopic sampling techniques has revolutionized the study of weaning in past populations. Isotopic techniques have been used for some decades to study weaning behavior by identifying characteristic changes in stable nitrogen and carbon isotopic ratios on a population-scale (Fogel, Tuross, & Owsley, 1989; Schurr, 1998; Wright & Schwarcz, 1998). These broad-scale studies, however, do not allow the archaeologist to look at individual life-histories and variation in weaning behavior. Incremental isotopic techniques now allow the bioarchaeologist to trace the weaning process on an individual level. Research using incremental isotopic techniques has shown it is possible to identify the onset, duration and cessation of the weaning process in past individuals (Beaumont, Gledhill, Lee-Thorp, & Montgomery, 2013; Beaumont & Montgomery, 2015, 2016; Beaumont, Montgomery, Buckberry, & Jay, 2015; Eerkens, Berget, & Bartelink, 2011). In addition, work is starting to relate incremental isotopic changes seen in clinical instances of starvation or nutritional deprivation (Fuller et al., 2005; Mekota, Grupe, Ufer, & Cuntz, 2006; Neuberger, Jopp, Graw, Püschel, & Grupe, 2013), to isotopic changes visible in archaeological populations experiencing the same stresses (Beaumont & Montgomery, 2016). Thus, by examining individual life-histories, incremental analyses allow us to potentially tease apart the effects of breastfeeding, weaning and physiological stress and to explore interactions between these factors. These individual stories can be combined to evaluate population-level hypotheses regarding female fertility or changes to population dynamics, whilst accounting for confounding factors, such as individual physiological stress. The ability to study childhood experience in tissues of individuals who survived childhood, alongside those who did not, also helps to test for mortality biases in weaning interpretations (i.e. the osteological paradox (Wood, Milner, Harpending, & Weiss, 1992)).

This study has two main aims. Firstly, to characterize the variation in early-life experience in prehistoric populations from the Atacama Desert, and assess whether there is a uniform shift in weaning behavior across the agricultural transition. Secondly, to identify isotopic patterns relating to weaning, and how these may be differentiated from those relating to early life

stress unrelated to diet. Prehistoric infant-feeding practices have not been well-studied in the Atacama Desert. Bulk isotopic analyses conducted further south in the Atacama Desert suggest that the pre-agricultural populations had developed a weaning practice which started early but was prolonged (with weaning generally lasting between 0.5 and 2.5 years) (Smith, Pestle, Clarot, & Gallardo, 2016). This early initiation of weaning, enabled through the use of marine resources as supplementary foods, potentially resulted in females becoming reproductively active again relatively quickly and therefore high fertility levels prior to agriculture in the region (Smith et al., 2016).

The study of weaning on an individual rather than a population level has not yet been attempted in this region. This is the first instance of incremental isotopic techniques' application to a prehistoric agricultural context in South America, and the first to compare pre-agricultural and agricultural populations. The majority of previous incremental isotopic analyses have been conducted using historic populations, and included comparison between historic sources, and events recorded in the isotopic profiles (Beaumont & Montgomery, 2016). In the populations of the Atacama Desert, background stress levels are likely to have been high simply because of the harsh desert environment, and high levels of infant mortality have been previously noted (Arriaza, 2005; Standen, Arriaza, & Santoro, 2014). We might, therefore, expect isotopic profiles to be dominated by evidence for environmental stress as well as reflecting infant-feeding practices. This context, therefore, provides us with the potential to identify a variety of different ways that stress may be visible isotopically in prehistoric samples, and how this may interact with the isotopic changes associated with the weaning process. It will provide general insight into how infant and child isotope results may be affected by life in the desert, and what may constitute 'normal' values in similar contexts. In addition, it will shed light on the process of agricultural adoption, and infant health in an extremely marginal environment.

In order to achieve the research aims we have three objectives: to obtain stable carbon and nitrogen isotopic profiles for 30 individuals from prehistoric sites of the northern Atacama Desert; to characterize the variability of these profiles; and to establish typical isotopic patterns and possible interpretations for these profile shapes. Results are presented and discussed with reference to *in utero* isotopic variation, followed by postnatal dietary changes. We focus on patterns in nitrogen isotope values, and patterns of negative covariance between the two isotopic systems, which we interpret as evidence for supplementary feeding and/or physiological stress.

METHODOLOGICAL BACKGROUND

The link between certain light stable isotope ratios and diet is well-accepted in archaeology (Ambrose & Norr, 1993; Lee-Thorp, Sealy, & Van Der Merwe, 1989). Nitrogen isotope ratios ($\delta^{15}\text{N}$) vary with trophic level of food source, and can thus be used to estimate the proportion of terrestrial meat consumption, or marine resource input into the diet (DeNiro & Epstein, 1981). Stable carbon isotopes in the terrestrial environment are fractionated on the basis of dietary plant photosynthetic pathway, with marine carbon also having a characteristic signal (Craig, 1953). Carbon isotopic ratios ($\delta^{13}\text{C}$) therefore tend to be used to differentiate terrestrial food sources from both one another and marine resources (Lee-Thorp, 2008). Traditional dietary isotope analysis involves the bulk sampling of bone, with isotopic ratios in this tissue representing 'average' diet over the period of bone formation/turnover, generally accepted to be 10 years or more in adults (Hedges, Clement, Thomas, & O'Connell, 2007).

Weaning as a process is visible isotopically in changes to both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, as it constitutes a dietary change (Fuller, Fuller, Harris, & Hedges, 2006a; Tsutaya & Yoneda, 2015). A breastfeeding child's $\delta^{15}\text{N}$ values will be elevated by 2-3‰ as they are, in effect, consuming their mother's protein (Fogel et al., 1989; Millard, 2000). As their diet is supplemented with weaning foods, or the supply of breastmilk decreases, their $\delta^{15}\text{N}$ values drop until they reach isotopic values typical of a post-weaning diet and weaning is considered complete (Fogel et al., 1989). Stable carbon isotope weaning curves typically echo those of nitrogen, as carbon isotopes also exhibit a trophic level shift of approximately 1‰ (Fuller et al., 2006a). The form of a hypothetical isotopic weaning curve is given in Figure 1. It is, however, worth noting that analytical uncertainties in isotopic analyses are typically around $\pm 0.2\text{‰}$ at 2 standard deviations. If the trophic level shift associated with breastfeeding and weaning is 2-3‰ in $\delta^{15}\text{N}$, contributions of weaning foods or breastmilk cannot be reliably detected when they are less than 7-10% of dietary protein. This means that weaning times established through isotopic methods are by no means absolutes, but weaning 'patterns' may still be compared and contrasted between individuals and populations.

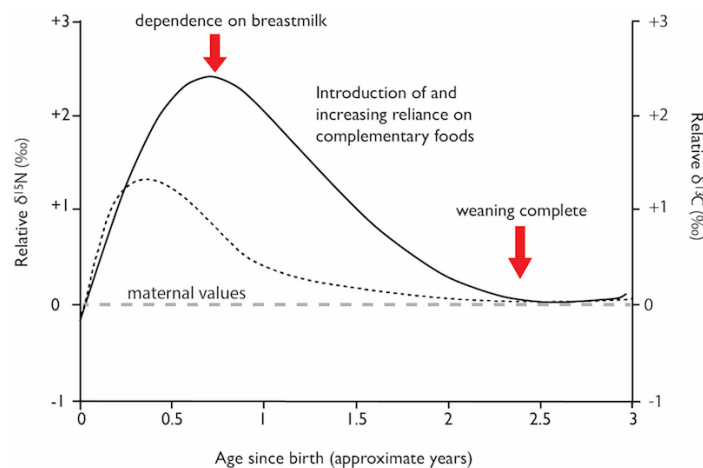


Figure 1: Schematic illustrating typical changes to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ during weaning for a population with a primarily terrestrial diet. Solid line represents $\delta^{15}\text{N}$, dashed line is $\delta^{13}\text{C}$ (adapted from Jay et al. (2008)).

To establish typical weaning time in archaeological populations, researchers may isotopically analyse bulk bone samples from infants and children of different ages. By identifying the individuals who have values closer to those of older post-weaning children and/or adults, and comparing their ages, it is possible to broadly identify when the population completes the weaning process (Jay, Fuller, Richards, Knüsel, & King, 2008; Millard, 2000; Schurr, 1998). Incremental isotopic analysis, on the other hand, involves the tracing of dietary changes through the lifespan of a single individual rather than at a population-level. This allows the researcher to examine detailed stories of early life dietary changes and compare individual experiences across the weaning transition.

Incremental analysis of dentine is based on the principle that this tissue forms at a known rate, and once formed does not remodel (Hillson, 1996; Nanci, 2007). This means that it can be sectioned into increments that represent a specific period of time during development (Beaumont et al., 2013; Beaumont & Montgomery, 2015; Eerkens et al., 2011; Henderson, Lee-Thorp, & Loe, 2014). Depending on the tooth chosen, and its formation times (Beaumont & Montgomery, 2015), it is possible to see dietary transitions during formation, which may

include weaning, or resource shifts during later childhood. In this study, we examine the weaning transition using incremental isotopic analysis focusing on teeth that form during early infancy and childhood. In analyzing both carbon and nitrogen isotopic ratios, which record different aspects of the diet, it may also be possible to differentiate different supplementary foods. Previous research has, for instance, interpreted differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trajectories as linked to supplementation of the diet with sheep or goat milk (Dupras, Schwarcz, & Fairgrieve, 2001; Sandberg, Sponheimer, Lee-Thorp, & Van Gerven, 2014), while unexpectedly elevated $\delta^{13}\text{C}$ values have also been interpreted as indicating maize gruel supplementation (Wright & Schwarcz, 1999).

Recent incremental isotopic research has resulted in the recognition that changes to isotopic ratios may not only reflect weaning but also periods of malnutrition or disease (Beaumont & Montgomery, 2016; Reitsema, 2013). Protein insufficiency, and sometimes caloric insufficiency, results in the catabolism of the body's existing proteins to meet nitrogen or energy requirements. Nitrogen isotopes within these catabolized proteins are essentially fractionated a second time leading to higher $\delta^{15}\text{N}$ in tissues formed during periods of protein catabolism (Fuller et al., 2005; Mekota et al., 2006; Neuberger et al., 2013; Reitsema, 2013). Periods of elevated $\delta^{15}\text{N}$ have been observed in modern studies of pregnant women suffering from morning sickness (Fuller et al., 2005), and anorexia nervosa patients (Mekota et al., 2006).

Stable carbon isotope ratios also have the potential to be altered in response to stress. Some studies have reported decreases in $\delta^{13}\text{C}$ corresponding with increases in $\delta^{15}\text{N}$ (Mekota et al., 2006; Neuberger et al., 2013). This phenomenon has been attributed to the breakdown of low $\delta^{13}\text{C}$ lipids to compensate for a lack of dietary carbohydrates and proteins which are normally the carbon source for tissue synthesis. This effect is not uniform, however, and some individuals show increases in $\delta^{13}\text{C}$ corresponding with increases in $\delta^{15}\text{N}$ (Neuberger et al., 2013). It is possible that this reflects utilization of different proportions of body protein and fat during starvation, due to differing amounts of body fat pre-starvation (Beaumont & Montgomery, 2016).

We can attempt to pinpoint episodes of stress by identifying isotopic patterns not conforming with those expected from the dietary changes of weaning. It may be possible, for instance, to identify maternal stress signals through elevated $\delta^{15}\text{N}$ values in the *in utero* forming increments of deciduous teeth (Beaumont & Montgomery, 2016; Beaumont et al., 2015). Similarly, stress signals from infancy or early childhood could show how and when the infant is mobilizing its own reserves to buffer against stress caused by the weaning process or external environmental factors (Reitsema, 2013). These isotopic techniques have the potential to identify periods of stress which cannot be seen using macroscopic or chemical analysis of bone. If the body mobilizes soft-tissue reserves to counteract the stress, or stress is recovered from quickly, then the slow turnover rate of bone will mask the chemical signatures of stress from these events (Reitsema, 2013). The precise time-resolution possible with incremental analyses, however, means that these short term changes to physiology may be visible.

The problem of equifinality in isotopic studies

All isotopic studies experience some difficulties in interpretation due to multiple pathways that lead to the same isotopic values or equifinality. Even considering dietary inputs alone, different proportions of terrestrial meat, marine resources, and C_3 and C_4 plants can result in the same isotope signatures. With the additional potential for stress-related changes to

isotopic values there are even more possible pathways to the values. For example, if a child's $\delta^{15}\text{N}$ values are raised relative to the adult population that could reflect exclusive breastfeeding, greater marine or terrestrial meat input into their diet, and/or episodes of protein insufficiency causing catabolism of their tissues. Different factors may also affect $\delta^{13}\text{C}$ changes. For example, a decrease in $\delta^{13}\text{C}$ in childhood could relate to either a change to more C_3 -dominated maternal diet transferred to the child through breastfeeding, the weaning process, or catabolism of lipids during a period of stress.

Even if isotopic changes can be definitively related to stress rather than diet, there are further potential interpretive issues. Protein insufficiencies may be caused through starvation, either due to lack of resource availability or because illness prevents the individual from eating. Illness itself encompasses both physical and psychological disorders. At the very most, therefore, isotopic results can give information about if/when stresses were experienced, and if they affected the body's protein or energy consumption.

Initial studies using incremental results have tended to focus on either clinical data or historic samples (Beaumont & Montgomery, 2016; Fuller et al., 2006a; Hatch et al., 2006; Mekota et al., 2006). In clinical studies, diet and stressors are well-described, and therefore isotopic patterns can be more easily linked to known life events. In historical contexts, records allow the characterization of the probable stress-environment experienced by the individual, giving more accurate interpretation of results (Beaumont & Montgomery, 2016; Henderson et al., 2014). In this study, however, we are applying these techniques to a prehistoric context with a relatively unknown stress environment. In doing so, we aim to highlight the multiple possible interpretations of incremental profile shapes, and explore whether equifinality issues can be overcome.

MATERIALS AND METHODS

Archaeological context of samples

This study assesses people from the pre-agricultural (Archaic) and agricultural (Formative – Late Period) sites of the Atacama Desert in Northern Chile (Figure 2). This area is one of the most extreme environments on Earth, comprising the driest hot desert in the world. Here rainfall is minimal and the main sources of water are the snowmelt-fed rivers (Lluta, Camarones and San Jose) that arise from the Andean cordillera. In sharp contrast to the inhospitable conditions of the desert, the marine environment is remarkably productive owing to the upwelling of the nutrient-rich, cold-water Humboldt current just off the coast (Blanco, Thomas, Carr, & Strub, 2001; Mohtadi, Romero, & Hebbeln, 2004).

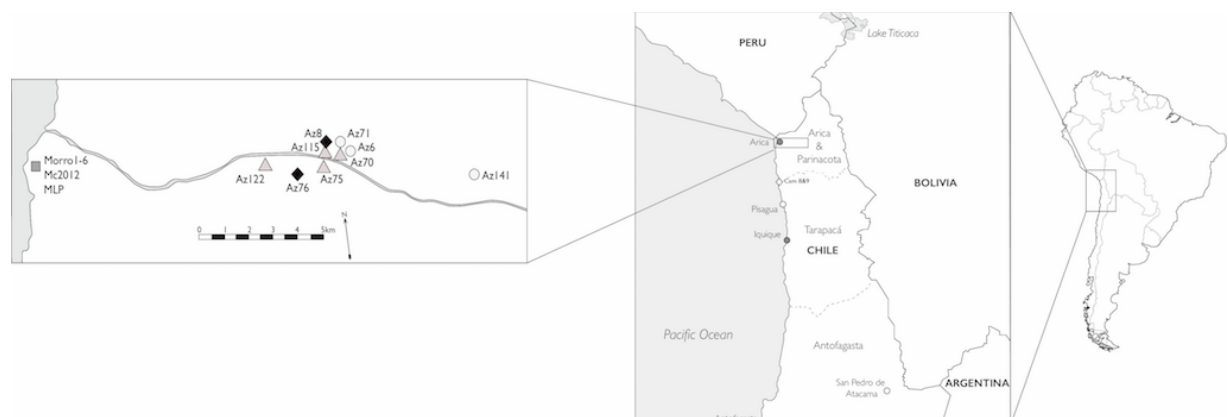


Figure 2: Location of the study area and sites sampled. Location information adapted from Sutter & Mertz (2004) and Muñoz & Zalaquett (2015).

The Archaic Period (4000-1700BC) in this region is characterized by the presence of the Chinchorro culture, complex hunter-gatherers whose subsistence relied heavily on marine resources. Agriculture begins in this area during the Formative period (1700BC-450AD), with Andean agricultural resources incorporated variably into a broad spectrum diet, focused on tubers (e.g. *Solanum*), gourds (*Cucurbita/Lagenaria*), pulses (*Phaseolus*), quinoa (*Chenopodium*), domesticated camelids (*Lama*) and some potential continued use of marine resources. Our previous isotopic research indicates that this variability in diet persists through much of the agricultural sequence, despite interactions with polities such as Tiwanaku in the Middle Horizon (450-900AD) and the Inka in the Late period (900-1450AD) whose economies were heavily based upon maize agriculture (Goldstein, 2003).

The study of prehistoric infants and children has been previously widely neglected (Halcrow & Tayles, 2008), and this is also true in our study area. The main exception in this region being the body of literature which focuses on high levels of infant mortality (Arriaza, 2005; Standen et al., 2014), attributed by some to heavy metals such as arsenic and lead in the river systems (Apata, Arriaza, Llop, & Moraga, 2017; Arriaza et al., 2010; Arriaza, 2005; Bartkus, Amarasiriwardena, Arriaza, Bellis, & Yañez, 2011). Recent research focusing on infant and child pathology at the sites in the present paper has reported high levels of metabolic disease and nutritional deficiencies, potentially linked to the vulnerability of desert populations to environmental instability, or parasitic infection resulting from high reliance on marine resources (Snoddy, Halcrow, Buckley, Standen, & Arriaza, 2017).

The timing of weaning is partly affected by resource availability, and in the generally resource-poor terrestrial environment of the northern Atacama it is possible that weaning time will be correlated with the availability of agricultural staples (although see Sellen and Smay, 2001 for a review of this concept). Many Andean staples can be used to make suitable weaning foods, and would have been available from the Formative period onwards (King et al., 2016; Pearsall, 2008, 2014; Smith, 1998). Today in traditional Andean societies such as the Aymará, quinoa is used to make gruel for weaning (Barton, Castro Williams, Barja, & Murillo, 2012). Similarly, tubers such as potato (*Solanum*), manioc (*Manihot esculenta*) or mashua (*Tropaeolum tuberosum*) may be boiled or mashed to form nutritious supplementary foods (Arbizu, Huamán, & Golmirzaie, 1997; Woolfe & Poats, 1987). Maize porridge has also been proposed as an easily digestible weaning food (Buikstra et al., 1986), and has been suggested as a potential trigger for population growth to the north in Peru, just prior to the emergence of polities in the Middle Horizon (Finucane, 2009). Although domesticated camelids were also present from the Formative Period onwards, there is no evidence that they were ever used for their milk and in fact, most Andean languages have no words for the drinking of animal milk (Gade, 1999).

Materials

This study sampled both permanent (n=5) and deciduous (n=25) teeth from sites spanning the entire prehistoric sequence in the region (Figure 2, Table 1). We sampled individuals from all archaeological periods, due in part to knowledge that preservation of collagen in early periods is variable (King et al., 2016). Previous isotopic work has also indicated that agricultural staples, such as maize, played a variable part in the adult diet through time (King et al., Early View; Pestle, Torres-Rouff, Hubbe, & Smith, 2016; Santana-Sagredo, Hubbe, &

Uribe, 2016; Santana-Sagredo, Lee-Thorp, Schulting, & Uribe, 2015; Santana-Sagredo, Uribe, Herrera, Retamal, & Flores, 2015), and it is likely that this variation also extends to childhood diet. By sampling widely, and including later archaeological phases, we increase the likelihood of identifying complementary feeding using maize, and the possibility of seeing changes to stress levels through time.

Deciduous molars were preferentially sampled, as the dentine horns of these teeth form *in utero* (Alqahtani, Hector, & Liversidge, 2010; Beaumont & Montgomery, 2015), and thus allow the evaluation of maternal isotopic values, potential for maternal stress and the impact this may have had on infant physiological stress. These teeth form between -0.2 and 3.5 ± 0.5 years (Beaumont & Montgomery, 2015), a time period that is likely to encapsulate the weaning process even in pre-agricultural societies, as multiple lines of evidence indicate that ‘traditional’ human weaning tends to be complete around 2.5 years of age, though there is well-documented inter-population variation (Dettwyler, 1995; Lewis, 2007; Schurr & Powell, 2005; Tsutaya & Yoneda, 2013). Sampling restrictions put in place by the Museo San Miguel de Azapa meant that fewer permanent teeth than deciduous could be taken for analysis. We preferentially sampled teeth which were already loose from their sockets. For permanent tooth sampling we preferentially sampled a tooth type which forms dentine during the typical weaning period, i.e. first molars (0.3 ± 0.13 yrs to 10 yrs ± 0.5 yrs) and canines (0.6 ± 0.15 yrs to 14.5 ± 0.5 yrs) (Beaumont & Montgomery, 2015). Details of the sample analysed are presented in Table 1. All infant and child age estimation was conducted using dental formation and eruption standards (Alqahtani et al., 2010), and adult age estimation (where possible) was conducted using composite scoring of the auricular surface (Lovejoy, Meindl, Pryzbeck, & Mensforth, 1985) and pubic symphysis (Brooks & Suchey, 1990), as well as late fusing epiphyses (Buikstra & Ubelaker, 1994). Adults are grouped into broad age categories (young, mid, old) rather than given specific ages as there are no population-specific aging standards currently available for the Atacama populations. Instead, young individuals fall into stages 1-2 of pubic symphysis and auricular surface scoring systems, mid individuals are categorized as stages 3-4, and old individuals are in stages 5+ (as per Buikstra & Ubelaker, 1994). Adult individuals without preserved pelvises were classified simply as ‘adult’.

Human remains from the Morro, Azapa Valley and Camarones sites are currently housed in the Museo San Miguel de Azapa (Arica, Chile), with the material from Maestranza Chinchorro and Maestranza La Pera sites deriving from salvage excavations conducted by Marco Portilla. This material is currently being accessioned into the collections at the Museo San Miguel de Azapa.

[Table 1 here]

Analysis

Incremental sampling was undertaken following method 2 of Beaumont et al. (2013). Teeth were half-sectioned longitudinally. Enamel was removed using a diamond cutting disk and retained for future analysis. Any secondary or tertiary dentine was removed using a dental burr (Beaumont et al., 2015). Half-sectioned teeth were demineralized in 0.5M HCl until the dentine was flexible. Dentine increments of 1 mm were cut using a sterilized surgical steel scalpel with a metal ruler for measurement, and placed into pre-weighed 1.5 ml microcentrifuge tubes for processing.

Collagen extraction followed a modified Longin method (Longin, 1971). Increments were gelatinized in pH 3 HCl in their microcentrifuge tubes on a hotplate for 24 hours at 75°C. After gelatinization, microtubes were centrifuged and the supernatant was lyophilized.

Isotopic analysis was undertaken in the Stable Isotope Biogeochemistry Laboratory (SIBL), Durham University. Total organic carbon, total nitrogen content and stable isotope analysis of the samples were performed using a Costech Elemental Analyser (ECS 4010) connected to a ThermoFinnigan Delta V Advantage isotope ratio mass spectrometer. Carbon isotope ratios were corrected for ^{17}O contribution and reported in standard delta (δ) notation in per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB). Isotopic accuracy was monitored through routine analyses of in-house standards, which were stringently calibrated against international standards (e.g., USGS 40, USGS 24, IAEA 600, IAEA N1, IAEA N2): this provided a linear range in $\delta^{13}\text{C}$ between -46 ‰ and $+3$ ‰ and in $\delta^{15}\text{N}$ between -4.5 ‰ and $+20.4$ ‰. Analytical uncertainty in stable carbon and nitrogen isotope analysis was typically ± 0.1 ‰ for replicate analyses of the international standards and typically < 0.2 ‰ on replicate sample analysis. Total organic carbon and nitrogen data was obtained as part of the isotopic analysis using an internal standard (Glutamic Acid, 40.82 % C, 9.52 % N). Collagen was assessed using Durham University Archaeology lab standards based on DeNiro (1985) and was considered to be of good quality if the C/N ratio was between 2.9 – 3.6, carbon weight % was between 35 – 50% and nitrogen weight % was between 11 – 16%.

Calculation of ages represented by increments

The chronological age represented by an incremental dentine sample was estimated using the method of Beaumont and Montgomery (2015). This takes into consideration the differing formation times of different teeth, differences in number of increments taken from each tooth, completeness of apex closure and, in the case of adult teeth, level of wear. This method allowed comparisons to be made between different individuals.

Differentiating isotopic signals representing weaning from those representing physiological stress

In previous studies, the weaning process has been defined isotopically as a 2-3‰ drop in $\delta^{15}\text{N}$ values, corresponding with a 1‰ decrease in $\delta^{13}\text{C}$ values (Burt & Garvie-Lok, 2013; Sandberg et al., 2014). This is equivalent to a trophic level shift, and implies that the infant has ceased consuming its mother's tissues. In a population with a relatively uniform post-weaning child and adult diet, the completion of weaning also involves the child's isotopic values becoming similar to the adult mean (Beaumont et al., 2015; Eerkens et al., 2011; Fuller, Molleson, Harris, Gilmour, & Hedges, 2006b). In the Atacama populations, however, adult diet is extremely heterogenous except in the Archaic and Late phases (King et al., Early View). In addition, it is likely that C_4 resources such as maize were introduced as supplementary foods during the weaning period, thus resulting in a departure from expected $\delta^{13}\text{C}$ values. We therefore examined changes to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values separately, and considered the 2-3‰ decrease in $\delta^{15}\text{N}$ values as primary evidence for weaning.

We followed the observations of Beaumont et al. (2015) and Beaumont and Montgomery (2016), to assign isotopic patterns to stress rather than weaning. Specifically, any changes to $\delta^{15}\text{N}$ which did not correspond to changes in $\delta^{13}\text{C}$, negative covariance between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, shifts in $\delta^{15}\text{N}$ of over 2-3‰ in magnitude (i.e. over one trophic level), and $\delta^{15}\text{N} > 2\text{sd}$

and >3 ‰ above the female mean, were considered possible evidence for physiological stress.

RESULTS

Individuals analysed in this study exhibit highly varied $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ profiles. Results are given in full in S1 Table, including collagen quality control indicators. Increments which did not yield enough collagen for analysis, or gave collagen values falling outside of acceptable parameters (n=47 of 378) were excluded from further analysis. Individual profiles of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for each individual are given as Supplementary figs 1-5. Figure 3 shows incremental dentine profiles for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, divided by phase to allow easier visualization of the data.

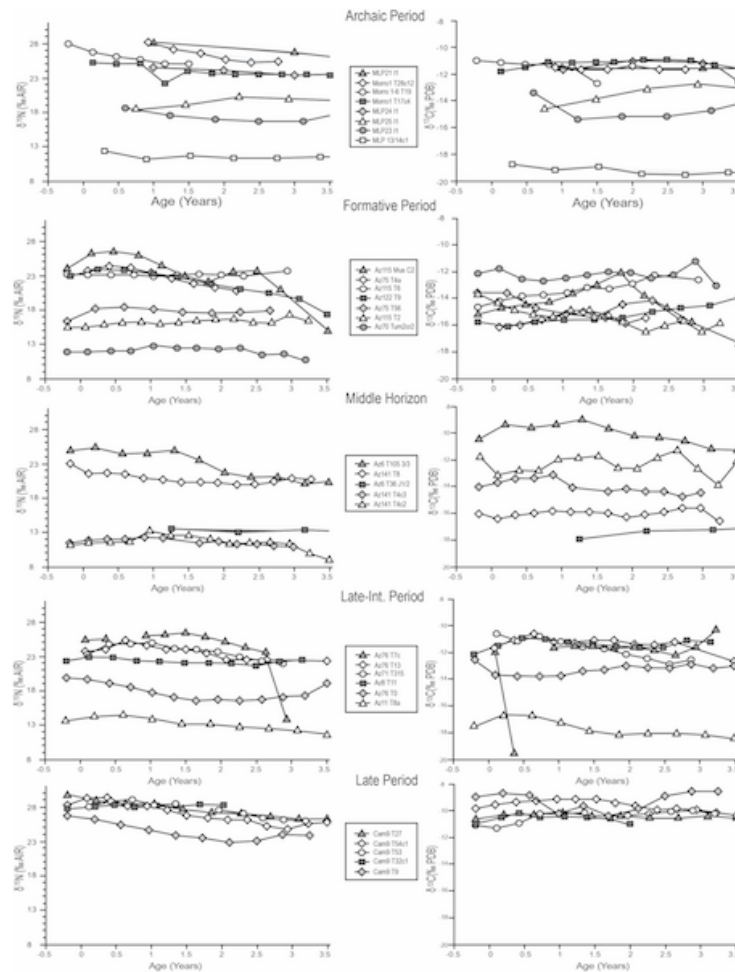


Figure 3: All incremental data obtained during this study, divided by archaeological phase. Left = $\delta^{15}\text{N}$ profiles, right = $\delta^{13}\text{C}$ values from the same individuals.

To assist in interpretation of the isotopic results we present our findings in three major parts, the first relates to values in the *in utero* forming increment; the second relates to postnatal $\delta^{15}\text{N}$ changes; and the third describes patterns of covariance in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values. Previous isotopic work is referred to throughout the results section, in order to place our results in context. We split the *in utero* values from the postnatal patterns because the *in utero* values are in essence the ‘starting point’ of each profile, and will therefore at least partially dictate the early postnatal values. Their variation and the reasons for it should, therefore, be interpreted first in order to provide a baseline from which the rest of the profiles may be

interpreted. For instance, if *in utero* values are high, then post-birth increments will also have high values, which may decrease more than is usual relative to ‘normal’ adult values.

In utero variation in isotopic values

Figure 4 shows isotopic values from the *in utero* increments of individuals in this study. Not all individuals had *in utero* values due to lack of analyzable material or tooth type sampled, but even with a reduced sample (n=20) size it is clear that the values of the ‘starting points’ for profiles vary widely. $\delta^{15}\text{N}$ values range between 11.2‰ and 28.4‰, while $\delta^{13}\text{C}$ values range between -17.5 and -8.9‰. Circles on Figure 4 represent clusters in the data established using k-means cluster analysis. Four clusters (k=4) were chosen to test whether there were differences corresponding to the time periods represented by our sample. In this analysis the Archaic and Late period were combined as previous bulk analysis has indicated that their baseline values are indistinguishable (King et al., Early View) and only one Archaic individual had *in utero* values.

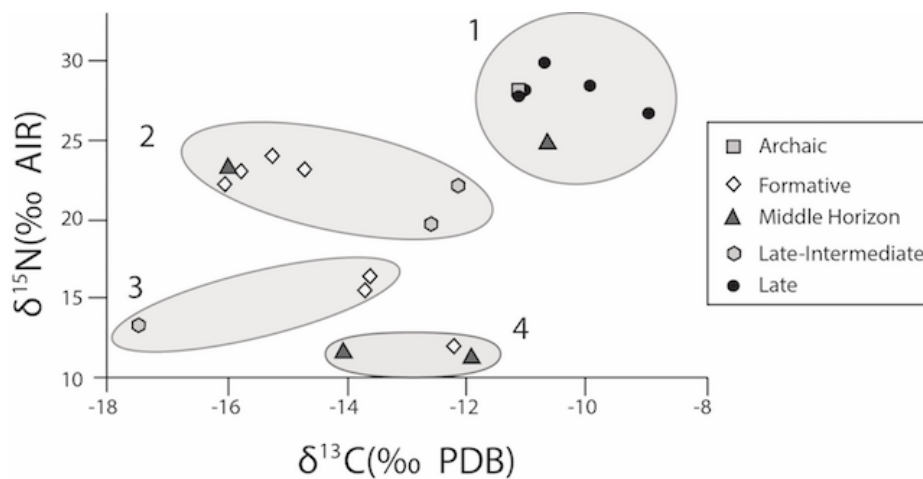


Figure 4: *In utero* forming increment isotopic values, shaded circles represent the groups identified by k-means cluster analysis

Although some clusters in the *in utero* values seem to relate to time periods, e.g., Cluster 1 includes all of the Late and Archaic Period individuals sampled, no cluster represents a single time period. *In utero* values, therefore, do not change systematically over time; rather they are variable throughout the archaeological sequence. In part, this *in utero* variation is likely due to maternal dietary variation. The clustering of these values aligns closely with our previous adult dietary isotope work in the area (King et al., Early View). The current paper’s incremental results show the grouping of coastal Archaic and Late Period individuals around high $\delta^{15}\text{N}$ values and more positive $\delta^{13}\text{C}$ values. This echoes the results of bulk isotopic analysis, and likely reflects the marine-resource consumption of mothers in these coastal sites. However, the inland sites of the Formative, Middle Horizon and Late-Intermediate period are characterized by very variable *in utero* increment values. Their wide-ranging $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values probably derive from the dietary heterogeneity seen in the adults during these time periods (King et al., Early View; Pestle et al., 2016; Santana-Sagredo, Lee-Thorp, et al., 2015; Santana-Sagredo, Uribe, et al., 2015).

In addition to maternal dietary variation, some of the isotopic variability in these *in utero* increments may also be caused by processes such as maternal stress, or physiological

differences. Changes to physiology during pregnancy may result in variable *in utero* $\delta^{15}\text{N}$ values. For instance, Fuller et al. (2005) noted increased maternal $\delta^{15}\text{N}$ values during periods of morning sickness, with a return to normal dietary values as gestation progressed. However, mothers experiencing physiological stress during their last trimester retained high values, resulting in high *in utero* $\delta^{15}\text{N}$ values (Fuller et al., 2005). In our sample it is difficult to establish what constitutes unusually high *in utero* $\delta^{15}\text{N}$ values, because adult diet in most archaeological phases is so variable (King et al., Early View), and adult female values in general may not necessarily reflect those of ‘healthy mothers’ (see Jay et al., 2008 for a review). Here we have used $>2\text{sd}$ and $>3\text{‰}$ above the female mean as parameters for defining values as ‘unusual’, and think it reasonable that values over this may indicate maternal stress, particularly during periods where diet was relatively homogenous e.g. Late and Archaic Periods. Of the individuals with *in utero* data 35% (7/20) fall into this category. Table 2 gives the isotope values of these individuals.

[Table 2 here]

This range in *in utero* isotope values illustrate that the exact isotope values for each individual will be heavily affected by different dietary baselines and maternal stress levels. Therefore, the comparison of isotope values between individuals in these population is not informative about dietary changes in infancy and childhood. It is only by comparing the shapes of isotopic profiles that inferences can be made about variation in life history.

Postnatal changes in $\delta^{15}\text{N}$

The classic weaning curve (Figure 1) predicts a decrease in $\delta^{15}\text{N}$ with weaning, and almost all individuals in the study sample exhibit some sort of decrease in $\delta^{15}\text{N}$, except Cam9 T32c1 and Az115 T6 who have flat $\delta^{15}\text{N}$ profiles, and Az115 T2 who has rising $\delta^{15}\text{N}$ values. There is, however, considerable variation in the magnitude and timing of these decreases (Table 3). Although the decreases are likely to be linked to weaning, their start and end points cannot be interpreted as corresponding precisely to the onset and completion of weaning. The isotopic signals are temporally smoothed by the turnover time of body protein (Millard, 2000) and, as noted above, small dietary proportions of supplementary food or breastmilk may not be detectable isotopically.

[Table 3 here]

The two individuals with flat $\delta^{15}\text{N}$ profiles are clearly unusual in this sample. Superficially their flat profiles could be interpreted as representing a lack of dietary change. In the case of infants and very young children this may mean the individual was unweaned at the time of death. However, beyond the age of 6 months supplementation of the diet is necessary to meet infant nutritional needs, and ensure survival (McDade & Worthman, 1998). In children older than one year, therefore, retention of exclusive breastfeeding behavior would result in quite extreme nutritional stress, resulting in a rise in $\delta^{15}\text{N}$ values as tissue catabolism occurs. We cannot, therefore interpret unchanging $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ beyond the age of around 1 year as a lack of weaning. The converse is also possible, that individuals with flat profiles were never breastfed at all. This is unlikely to have been a common scenario, but if a mother died in childbirth, or close to the time of birth and no other lactating adult was available infants would have had to have been placed onto a non-breastmilk diet immediately. A study of mummified female remains from the Azapa Valley has highlighted the presence of fetal or placental remains still within the uterus in 18% of young females (Arriaza, Allison, &

Gerszten, 1988). This has been used to suggest that complications during or just after childbirth were relatively common and often resulted in maternal death, making a lack of breastfeeding in these populations a possibility. Again, this process is likely to have involved significant stress to the infant as its under-developed digestive system attempted to process harder to digest foodstuffs and was potentially exposed to pathogens, but some individuals could have survived this process, at least for a time (e.g. Moucheraud et al., 2015).

Given that one of the two individuals with flat profiles (Cam9 T32c1), is the youngest individual included in our sample (18 months) it is possible that their unchanging $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values indicate a real lack of dietary change and they were either never breastfed or exclusively breastfed for the duration of their short life. It is possible that this was also the case for Az115 T6. In the complete absence of breastfeeding, however, infant survival beyond the first few months of life is unlikely (Knodel & Kintner, 1977), particularly without animal milk to act as a substitute (see introduction). In the case of Az115 T6 we consider it more likely that they weaned onto a higher trophic level food source than was typical for the maternal diet, thus masking the trophic level drop normally associated with the weaning process. This interpretation is also supported by changes to $\delta^{13}\text{C}$ values in this individual, which increase from around 8 months of age, perhaps indicating the introduction of this weaning diet. A dietary shift to high trophic level resources may also be responsible for the rising $\delta^{15}\text{N}$ values in Az115 T2, but in this instance $\delta^{13}\text{C}$ values decrease as $\delta^{15}\text{N}$ values increase, making physiological stress a potential causative factor. This negative covariance of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values is discussed below.

Four individuals display $\delta^{15}\text{N}$ values which begin to decrease from birth, rather than remaining elevated as is typical for the period of breastfeeding: Cam9 T27 (Figure 5), Morro1-6 T19 (S1 fig), Az76 T0 (S4 fig), and Az141 T8 (S3 fig). This pattern was identified by Beaumont et al. (2015) in their study of Irish famine victims, and was interpreted as being fundamentally linked to high *in utero* $\delta^{15}\text{N}$ values. In that study, it was proposed that postpartum maternal dietary change could be mimicking the trophic level shift of weaning much earlier than expected. For instance, if the mother switches to a lower trophic level diet after giving birth then their $\delta^{15}\text{N}$ values would decrease, and this decrease would be transmitted to the infant through breastmilk. It is also possible that if the infant was in a stressful *in utero* environment, birth may reduce that stress as the infant is no longer so intrinsically linked to maternal physiology. This would result in an initial lowering of $\delta^{15}\text{N}$ values unrelated to dietary change. In our sample most of the individuals with $\delta^{15}\text{N}$ values decreasing immediately post-partum also have high *in utero* $\delta^{15}\text{N}$ values (see previous section), supporting the interpretation of Beaumont et al. (2015) that this pattern is caused by high maternal stress.

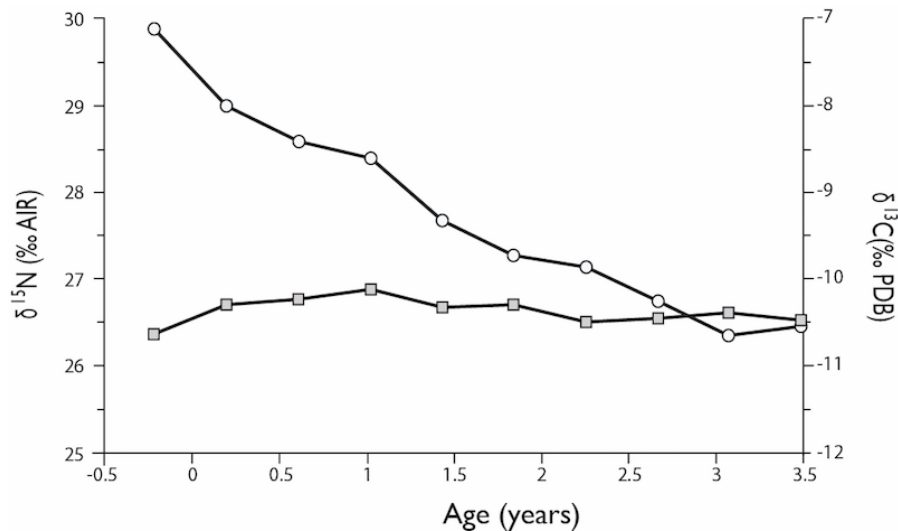


Figure 5: Example of an incremental profile (Cam9 T27) which exhibits decreasing $\delta^{15}\text{N}$ values from birth.

Finally, it is also possible that the decrease in $\delta^{15}\text{N}$ from birth relates to a complete lack of breastfeeding, and immediate feeding with lower trophic level foods than were typical for the adult diet. However, if this were the case we would expect a step-like shift in values reflecting an abrupt change in diet. These profiles exhibit gradual curves, similar to those seen in normal weaning, but beginning immediately after birth, suggestive of a gradual process.

Some individuals exhibit decreases in $\delta^{15}\text{N}$ values postnatally that are larger than expected for just one trophic level shift (normally 2-3‰), while others have a smaller than expected shift, some of only around 1‰. These discrepancies may represent different interactions between weaning, maternal or infant dietary change and physiological stress. For example, both De Luca et al. (2012) and Fuller et al. (2006a) report infant values that are up to 1‰ higher than maternal values at birth. This has been interpreted as being due to temporary changes in maternal diet or physiology during the early postnatal period (Beaumont et al., 2015).

Some individuals fall just outside the expected decrease in $\delta^{15}\text{N}$ values associated with a weaning shift, for example Az6 T105 3/3 (5‰) and Cam 9 T54c1 (5.4‰). These changes fall within some of the more extreme published values for a single trophic level decrease (Caut, Angulo, & Courchamp, 2009). Therefore, these profiles may simply represent a classic weaning trajectory with slight interactions with stress or physiological differences in the mother-infant pairs causing slightly larger than normal drops in $\delta^{15}\text{N}$ values.

Other individuals have decreases in $\delta^{15}\text{N}$ values which fall well outside of normal weaning parameters. Az122 T9 decreases by 6.5‰, and Az115 Mus C2 and Az76 T7c both drop by 11.7‰. In these instances, it is likely that a combination of weaning and maternal/infant dietary change are causing these very large shifts. In both Az122 T9 (Figure 6) and Az115 Mus C2 (S2 fig) isotopic profiles seem to follow a normal weaning curve initially, dropping smoothly by 3‰ between 6 months and 2.5 years. It is only after this point that $\delta^{15}\text{N}$ values decrease sharply to the much lower values found at the end of tooth formation. Though some data are missing for Az76 T7c (S4 fig), a gradual and minor decrease in $\delta^{15}\text{N}$ values seems to be complete by 2.5 years of age, at which point $\delta^{15}\text{N}$ values drop off steeply and $\delta^{13}\text{C}$ values

begin to increase. In all three of these individuals their isotopic curves could be evidence for the completion, or near-completion of a normal weaning process, and then a sharp shift to a lower trophic level diet, perhaps with more C₄ plant input causing a corresponding increase in $\delta^{13}\text{C}$ values.

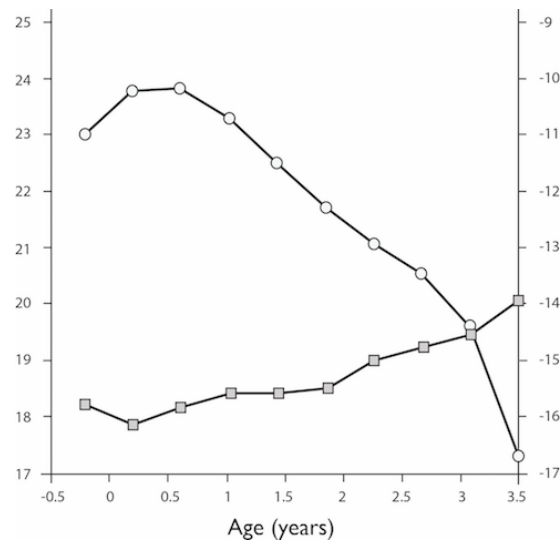


Figure 6: Example of an incremental profile (Az122 T9) exhibiting a decrease in $\delta^{15}\text{N}$ values of over one trophic level.

Individuals with a smaller decrease in $\delta^{15}\text{N}$ values than predicted by the classic weaning curve may also be affected by dietary differences between the mother and infant during the weaning process. For example, Az8 T11 (S4 fig) follows what appears to be a normal weaning curve with both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values decreasing gradually between 6 months and 2 years of age. Beyond this, however, both curves begin to rise to higher values again. It is possible that this indicates that the final stages of weaning involved greater input from higher trophic level supplementary foods than were present in the maternal diet (as reported by King et al. (Early View)), causing a decrease in $\delta^{15}\text{N}$ values of lower magnitude than expected. Alternatively, the increase in $\delta^{15}\text{N}$ values caused by physiological stress may be effectively ‘cancelling out’ the decrease which would normally be visible as the weaning process occurs.

Covariance of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values

The majority of individuals in our sample have profiles which follow the expected pattern of positive covariance between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values at least in the initial portion of the isotopic profile. This pattern reflects the weaning trophic level decrease which should be recorded in both isotopic systems. There are, however, instances where carbon and nitrogen isotope values negatively covary for part of the profile. Particularly common is the pattern of decreasing $\delta^{15}\text{N}$ values, as expected during the weaning process, but with increasing rather than decreasing $\delta^{13}\text{C}$ values (Figure 7). This occurs in seven of the individuals analysed (Morro1 T17c4, Az115 Mus C2, Az75 T4a, Az75 T56, Az76 T0, Az76 T7c and Cam9 T53).

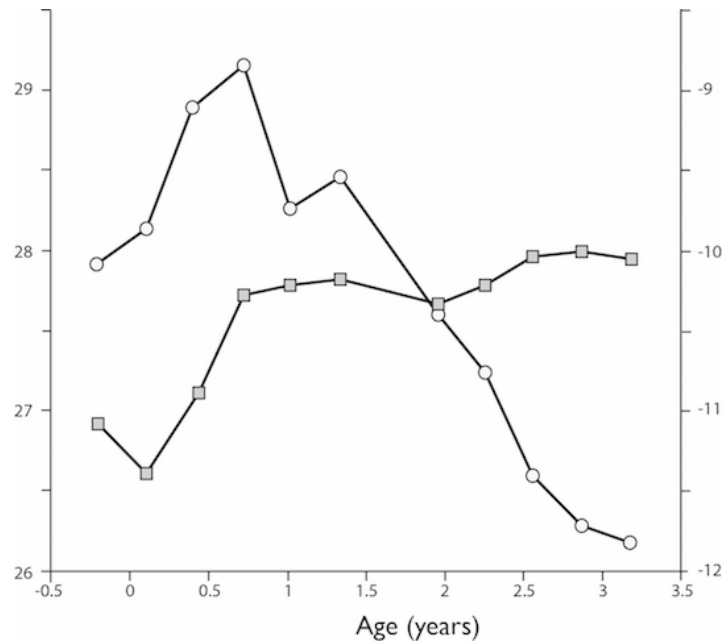


Figure 7: Example of an incremental profile (Cam9 T53) showing negative covariance between falling $\delta^{15}\text{N}$ values and more positive $\delta^{13}\text{C}$ values from the age of approximately 1.5 years.

This pattern may be linked to dietary change, either introduced to the infant directly or through changes in the isotopic composition of breastmilk. For instance, if an infant was being weaned onto resources with more positive $\delta^{13}\text{C}$ values than those in the maternal diet we would see a rise in $\delta^{13}\text{C}$ values relative to the mother's milk, effectively 'cancelling out' the trophic level shift expected during weaning. Depending on the proportion of C_4 resources to breastmilk, and the isotopic differences between the supplementary food and the mother's diet this could result in either a flat carbon profile or $\delta^{13}\text{C}$ values which gradually increase as more supplementary food and less breastmilk is consumed. This pattern has been observed in previous studies (Sandberg et al., 2014; Wright & Schwarcz, 1998), and has been linked to the use of C_4 foods such as maize as supplementary resources during agricultural periods. This is feasible in the Atacama context from the Formative period when maize agriculture was introduced to the area, but is not possible for Morro1 T17c4 (S1 fig) as during the Archaic period C_4 resources were not available. Our adult dietary isotopic work has shown that, particularly in the Formative and Late-Intermediate periods, maize was only variably incorporated into the adult diet, thus its use as a weaning food would result in $\delta^{13}\text{C}$ values that became more positive than the adults' during the weaning process. It is, however, equally possible that this pattern could reflect gradual changes to the maternal diet transmitted to the infant through breastmilk. In the case of Morro1 T17c4, the rise in $\delta^{13}\text{C}$ values is only slight (less than 1‰), a change which could reflect a shift in maternal diet to different marine resources. In this area, marine plants can have $\delta^{13}\text{C}$ values up to 1‰ higher than marine fish and mammals (King et al., Early View). It is therefore possible that the pattern seen in Morro1 T17c4 represents either supplementation with marine plants, or a maternal diet becoming more dominated by marine plants.

There are other instances of negative covariance in this sample, however, where $\delta^{15}\text{N}$ values spike as $\delta^{13}\text{C}$ values decrease sharply. These are short-lived changes which do not echo the weaning curve. For example; Az141 T4c2 (-0.5-0.1yrs and 3.2-3.5yrs), Az15 T6 (2.5-2.9yrs),

Az122 T9 (-0.5-0.2yrs) and Az75 T4a (-0.5-0.1yrs). These spikes may represent temporary dietary changes, but it is perhaps more likely that they are recording episodes of physiological stress, where the body is catabolizing lipids in order to maintain homeostasis. The body has multiple energy stores it can access to mitigate the effects of prolonged nutritional deprivation, and while catabolism of proteins may cause both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values to increase, catabolism of ^{13}C depleted lipids (Kelly, 2000) is likely to cause a decrease in $\delta^{13}\text{C}$ values associated with an increase in $\delta^{15}\text{N}$ values. It is difficult to disentangle the effects of diet from those of lipid catabolism, but we feel that lipid catabolism is the most parsimonious explanation for the pattern in some of our individuals. For instance, Az115 T6 has a sharp spike in $\delta^{15}\text{N}$ values close to time of death which corresponds with a sharp decrease in $\delta^{13}\text{C}$ values. While it is possible that this relates to a swift dietary change, the proximity to time of death lends itself to the interpretation that this change represents a stress episode. Similarly, increases in $\delta^{15}\text{N}$ value around birth which correspond with a decrease in $\delta^{13}\text{C}$ values (e.g. in Az141 T4c2, Az122 T9 and Az75 T4a), may relate to postnatal physiological changes, perhaps including stress during the early months of infancy.

DISCUSSION

Timing of the weaning process and its relationship to the presence of agricultural staples

Our results highlight the variable early-life experiences of infants and children in the Atacama Desert. Models of agricultural development generally predict changes to weaning behavior with the introduction of agricultural staples (Armelagos, Goodman, & Jacobs, 1991; Bocquet-Appel, 2011; Buikstra et al., 1986), but this does not appear to be the case in the northern Atacama. Changes to $\delta^{15}\text{N}$ values indicate that individuals even within the same archaeological phase began and completed weaning at different times. In the studied sample, differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values between individual profiles seem to be primarily based upon adult (i.e. maternal) dietary differences. This maternal dietary variation is significant and results in a spread of infant isotopic values. In this respect, these incremental results replicate the bulk isotopic results from the same archaeological sites (King et al., 2016). It appears that environmental constraints dictated both the diet of adults and the supplementary food given to children, resulting in retention of broad-spectrum habits despite the presence of agricultural resources. These isotopic findings align well with previous archaeological interpretations, which highlight the continuation of material culture associated with marine resource exploitation (Muñoz, 2004), alongside evidence for Andean cultivars (Erices, 1975) and the growing symbolic importance of domestic camelids (Valenzuela et al., 2015) and maize (Goldstein, 2003). Our results also align well with previous bulk isotopic analyses which suggest early and prolonged weaning in the Archaic populations of the region, and hinted that because of this, fertility was already high and a demographic transition with the incoming of agriculture was unlikely (Smith et al., 2016).

While our results do not suggest any change in weaning behavior through time, it is important to note that the small sample size, and long occupation period of some of the archaeological sites analysed may be masking changing patterns in weaning behavior. We could, for example, be mistaking inter-generational change in behavior for idiosyncratic weaning choices. With larger sample sizes or better temporal constraint on the samples it is possible that some systematic differences in behavior could emerge. This study therefore paves the way for future research examining whether the variation in this sample is truly the result of idiosyncratic behavior, or if it may be reflecting other processes.

The use of agricultural crops as weaning foods

This study gives new insight into the potential importance of agricultural crops as weaning foods. Although previous isotopic work suggested that C₄ resources were only variably incorporated into the adult diet (King et al., Early View), incremental results show that some children relied upon C₄ resources as supplementary foods during the weaning period. There is also evidence for childhood diet rapidly diverging from adult diet in terms of steep decreases in $\delta^{15}\text{N}$ towards the end of tooth formation in some individuals (Az115 Museo C2, Az76 T7c and possibly Az122 T9). In these instances, it appears that children, once weaned, were switched to a lower trophic level diet, probably involving terrestrial agricultural resources. These patterns, however, do not appear to be strongly linked to archaeological time period. Individuals with evidence for maize gruel consumption, and those who seem to have experienced a dramatic shift in diet around the end of tooth formation are not constrained to one archaeological phase.

The influence of physiological stress

In addition to recording dietary variation in early life, the incremental profiles obtained in this study also highlight the influence of physiological stress. Multiple individuals have *in utero* isotopic values which fall well outside the norm for their phases, indicating the possible influence of maternal stress. Many of these individuals also display a decrease in $\delta^{15}\text{N}$ values which begins at birth, instead of the expected maintenance of high values during exclusive breastfeeding. We interpret this as relating to changes to postnatal physiological stress levels as the infant is removed from the stressful *in utero* environment. In addition to these features, there is also isotopic evidence for acute periods of stress in the negative covariance of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in some individuals. While it is hard to unequivocally interpret the presence of stressors, in some cases the most parsimonious explanation for our isotopic data is protein or caloric insufficiency and tissue catabolism. This interpretation is supported by paleopathological evidence from some of the sites studied here, which highlights the presence of lesions associated with metabolic bone disorders, including scurvy (Snoddy et al., 2017). This suggests that the prehistoric populations of the Atacama were subject to periodic episodes of resource insecurity and associated micronutrient deficiencies. The presence of these lesions in young infants indicates that these dietary insufficiencies were dramatic enough to be transferred to the infant *in utero* (Snoddy et al., 2017).

Isotopic evidence for stress is present throughout the archaeological sequence and does not appear to change with the arrival of agriculture or different cultural groups in the area. There are individuals with high *in utero* $\delta^{15}\text{N}$ values in the pre-agricultural Archaic period, incipient agricultural Formative Period and state society of the Late Period. Similarly, individuals with evidence for acute episodic stress are present in all time periods. In this desert context, stress is likely to have been a relative constant. The area is marginal agricultural land, and has been at the mercy of the El Niño Southern Oscillation (ENSO) since the beginnings of the current regime around 5000BP (Williams, Santoro, Smith, & Latorre, 2008). This interpretation of high levels of stress during prehistory is supported by paleopathological evidence for dietary insufficiencies (Snoddy et al., 2017), and evidence for exposure to heavy metals in river water throughout prehistory (Arriaza et al., 2010), and the commonplace nature of parasitic infection, probably due to heavy reliance on marine resources (Arriaza, Reinhard, Araújo, Orellana, & Standen, 2010; Santoro, Vinton, & Reinhard, 2003). These environmental

stressors remain constant throughout the archaeological sequence. While the introduction of agricultural resources may have slightly buffered the population against resource instability, extreme ENSO cycles, the presence of parasites and environmental contaminants remained out of their control.

Summary

These data provide important insight into how incremental isotope results may be affected by the presence of environmental stressors and the use of supplementary foods. The patterns in our data, which may relate to *in utero* and postnatal stress and complementary feeding, will provide important baseline data for interpretation of future incremental work. In the Atacama much is yet to be done in terms of osteological analysis of the desert populations. Ideally our isotopic results should be combined with paleopathological analysis of the same individuals, examining whether possible stress signals evident via tooth chemistry are visible macroscopically in the bones. Future studies may also look at the morbidity and mortality outcomes of the variable infant-feeding practices in the Atacama sample to establish whether there are differences between survivors and non-survivors.

CONCLUSION

The data presented here highlight the variability of early-life experiences among the prehistoric inhabitants of the Atacama Desert. It is the first sample in South America to be analyzed using incremental isotopic techniques, and allows us unprecedented insight into infant-feeding practices. Although there is evidence for weaning in most of the isotopic profiles obtained in this study, there is considerable variation in the dietary baseline of individuals, timing of the weaning process, and the use of certain agricultural staples as weaning foods, as well as evidence for physiological stress. This variation is not systematic, and there are no trends in weaning practice over time; instead individuals within the same sites and phases appear to have been following very different infant feeding patterns. The variability of these isotopic results is likely to be a product of the archaeological context. The Atacama Desert is one of the harshest environments on Earth. Here, the environmental conditions are so marginal and food sources so vulnerable, that it is likely that mothers mitigated stressors by following individualistic infant-feeding trajectories tailored to the environment at the time and infant health, among other factors.

These results highlight the uses of incremental isotopic analysis in prehistoric contexts. While bulk isotopic analyses in the area have highlighted a lack of agricultural reliance in adults, incremental results reveal the importance of agricultural staples as complementary foods during weaning. The high time-resolution of incremental analyses also allows the bioarchaeologist to identify short-term physiological changes, visible as *in utero* and postnatal stress signals. Interpretation of incremental results in a relatively unknown prehistoric context remains difficult, and we also highlight the equifinality issues inherent in our results. Future studies should consider how to combine incremental isotopic results with paleopathological data to create the most parsimonious explanations of isotopic patterns.

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